PROC. ENTOMOL. SOC. WASH.
98(1), 1996, pp. 126–137

COURTSHIP BEHAVIOR, REPRODUCTIVE RELATIONSHIPS, AND ALLOZYME PATTERNS OF THREE NORTH AMERICAN POPULATIONS OF *ERETMOCERUS* NR. *CALIFORNICUS* (HYMENOPTERA: APHELINIDAE) PARASITIZING THE WHITEFLY *BEMISIA* SP., *TABACI* COMPLEX (HOMOPTERA: ALEYRODIDAE)

MARTHA S. HUNTER, MICHAEL F. ANTOLIN, AND MIKE ROSE

(MSH) Assistant Research Scientist, Department of Entomology, Texas A&M University, College Station, TX 77843, U.S.A.; (MFA) Assistant Professor, Department of Biology, Colorado State University, Fort Collins, CO 80523, U.S.A.; (MR) Research Scientist, Department of Entomology, Texas A&M University, College Station, TX 77843, U.S.A.

Abstract.—Throughout the southern U.S., the serious whitefly pest, *Bemisia* sp. is parasitized by morphologically similar aphelinid parasitoids that have all been called *Eretmocerus* nr. *californicus*. In this study, the courtship behavior, reproductive compatibility, and allozyme patterns of three populations of *E.* nr. *californicus* from Texas, Arizona, and California were investigated to determine their species status. The courtship behavior of the three populations did not differ in the kinds of behaviors performed; the sequence of behaviors, or the frequency or duration of behaviors. Reciprocal mating trials between *E.* nr. *californicus* from Texas and *E.* nr. *californicus* from Arizona or California did not result in any successful mating, but there was no significant difference between the frequency of between- and within- population matings in the Arizona and California populations. Isoelectric focusing electrophoresis for the 3–5 allozyme loci scored for each population showed divergence of the Texas population from the populations from Arizona and California. The allele frequencies at the IDH locus differed between the Texas and the Arizona and California populations. Similarly, allele frequencies at the ME locus differed between the Texas and the Arizona populations. The Texas population showed two unique alleles at the MDH locus, but otherwise MDH, PGI, and PGM showed similar allele frequencies in all populations. The results of the mating trials and electrophoretic study corroborate subtle morphological and pigment differences between the Texas population and Arizona and California populations, and it is concluded that these populations represent two species.

Key Words: Systematics, electrophoresis, biological control, sweetpotato whitefly, silverleaf whitefly

Systematists involved in biological control projects may be confronted with an array of morphologically similar natural enemies from different geographical sources. Accurately delineating species is of critical practical importance; classifying a group of similar populations as the same species when they are not may obscure life history differences, and prevent effective natural enemies from being introduced to a new habitat (Rosen and DeBach 1973, Rosen 1986). Sibling species may differ in such

important characteristics as their host or habitat affinities (Rosen 1986, Wharton et al. 1990). Conversely, making spurious distinctions among conspecifics may result in unnecessary introductions and confusion in evaluating biological control efforts (Rosen 1986). Biosystematic studies of reproductive relationships among populations may add to our understanding of species limits within a particular group, and more generally, to our knowledge of how variation in different character systems such as morphology, life history, behavior, and biochemical or genetic markers are correlated.

Widespread and damaging outbreaks of a whitefly, *Bemisia* sp., in many cropping systems of the Southern United States from 1986-1991 prompted a national research effort. Originally called the "B strain" of *Bemisia tabaci* (Gennadius), the whitefly has recently been given species status, and has been named *Bemisia argentifolii* Bellows and Perring, the silverleaf whitefly (Bellows et al. 1994). Controversy over the identity of this whitefly remains, however (see e.g. Bartlett and Gawell 1993, Campbell et al. 1993, Brown et al. 1995), therefore we will refer to it as *Bemisia* sp., *tabaci* complex.

Surveys of natural enemy species of this whitefly species have demonstrated that many if not all of the endemic natural enemies recorded from the sweetpotato whitefly attack the silverleaf whitefly (Gerling 1966, Coudriet et al. 1986, Polaszek et al. 1992, McAuslane et al. 1994). In all of these surveys, parasitoids in the genus *Eretmocerus* have been found, as well as one or more species of *Encarsia.* In southern California and Arizona, *Eretmocerus* dominates the parasitoid complex, and the *Eretmocerus* species in Texas is dominant at some times of the year (M. Rose, J. Woolley, and C. Moomaw, in litt.). In Florida as well, *Eretmocerus* may sometimes dominate (L. Osborne, pers. comm.). The identities of these *Eretmocerus* populations have been unclear. They have all been called *Eretmocerus* nr. *californicus,* but subtle morphological differences separate them from *Eretmocerus californicus* Howard (1895) (M. Rose and G. Zolnerowich, in litt.), originally described from an unknown whitefly on *Quercus agrifolia.* Small differences in sculpturing, antennal configuration, wing shape and setation of the females, and pigmentation of the males, differentiate wasps collected from Arizona and California from those from Texas, and the apparently mixed Florida population from either of these two groups (M. Rose and G. Zolnerowich, in litt.).

In order to interpret the biological meaning of these small morphological differences, we studied the reproductive relationships and allozyme patterns among three populations of *Eretmocerus* collected from *Bemisia* sp. in Arizona, California and Texas. We were also interested in evaluating the courtship behavior of these populations for the presence of distinguishing behavioral characters, and in a broader context, to compare courtship in this genus with courtship described for other aphelinid genera such as *Aphytis* (Gordh and DeBach 1978), and *Encarsia* (Viggiani and Battaglia 1983, Kajita 1989).

Currently, indigenous and exotic species of *Eretmocerus* are being evaluated and colonized for biological control of *Bemisia* sp. in the U.S. In addition, the *Eretmocerus* sp. collected from *Bemisia* sp. in Texas has been reared and released in California (C. Pickett, pers. comm.). Another objective of this study was to determine the likelihood of these populations hybridizing when sympatric, and to develop character systems which could be used in addition to morphological characters to aid in distinguishing the naturally occurring populations of *Eretmocerus.*

Materials and Methods

Collection information.—Three *Eretmocerus* populations were sampled in this study. In Weslaco, Texas, cabbage leaves bearing parasitized *Bemisia* were collected on June 10, 1993. Melon leaves bearing

parasitized *Bemisia* were collected in Bard, California on June 7, 1993, and poinsettia leaves bearing parasitized *Bemisia* were collected in a greenhouse in Tucson, Arizona on June 11, 1993. The greenhouse supported a large continuous culture of an *Eretmocerus* that was originally collected from *Bemisia* in Phoenix, Arizona (O. Minkenberg, pers. comm.). All of the leaves were sent to College Station, where approximately 300–400 pupae from each collection were isolated in ¼ dram vials, and held in a humidified environment until emergence. These individuals were used for the mating trials, and for electrophoretic study. In May of 1994, a second collection of *Eretmocerus* was made from the Rio Grande Valley in Texas and the same greenhouse in Tucson, Arizona for further electrophoretic study.

Mating trials.—Small Syracuse watch glasses (2 cm diam.) covered with a microscope slide were used as arenas in which to observe courtship and mating. The watch glasses were lined with a collard leaf disk bearing *Bemisia* nymphs. Females 24–48 h old were introduced to the arenas, and then males of similar age were introduced. The behavior of the wasps was observed using a dissecting microscope at 8× for 10 minutes, or until completion of a successful mating. A video camera was attached to the dissecting microscope and the behaviors videotaped for further analysis.

Females that were successfully mated were isolated on small caged collard plants bearing *Bemisia* nymphs. The pupal progeny were then collected and the adults sexed at emergence.

Allozyme analysis.—Samples of field-collected wasps from each of the three field populations and a sample of F1 and F2 progeny from successful matings were frozen in liquid nitrogen for allozyme analysis. Electrophoresis was performed by isoelectric focusing (IEF) of enzymes on 11 cm wide cellulose acetate membranes (Fuji Separax-EF, Wako Chemicals, Richmond), using the apparatus and methods described by Kazmer (1991) for three electrodes and an effective membrane size of 4.5 cm. Staining of cellulose acetate membranes for allozymes was according to standard recipes, with the stains applied in 0.75% (wt/vol) agar overlays (Bacto-agar, Difco Laboratories, Detroit).

Wasps from the three populations used in mating tests were initially screened in IEF runs using carrier ampholytes (Sigma Chemical, St. Louis) that create a pH 4–6.5 gradient between the cathode and anode. Insects were squashed directly onto membranes 1.8 cm from the cathode. By stacking three membranes, we were able to stain and resolve three polymorphic allozymes (isocitrate dehydrogenase, IDH; malate dehydrogenase, MDH; glucose-phosphate isomerase, PGI), with each membrane stained for one enzyme. F1 and F2 progeny from crosses were also electrophoresed to verify Mendelian segregation of alleles. A second run was carried out using wasps from second collections of wasps from the Texas field population and the Arizona greenhouse population. This time, carrier ampholytes that create a pH 5–8 gradient were used, which allowed us to resolve two additional enzymes (malic enzyme, ME; phosphoglucomutase, PGM). In this case, four membranes were stacked and stained, with one of the four membranes stained for both ME and MDH.

Alleles were identified by their distance from the cathode, with the most cathodal allele in each case designated the "a" allele. After genotypic data for each locus were recorded from stained membranes, population statistics and Nei's (Nei 1972) genetic distance were calculated by entering the data into BIOSYS-1 (Swofford and Selander 1989). In addition a modification of Nei's D that is less sensitive to variation among loci in gene substitution rates, D*, was calculated (Hillis 1984). Tests of Hardy-Weinberg genotypic proportions were carried out on data from females only, while pairwise measures of genetic distance between populations were calculated from

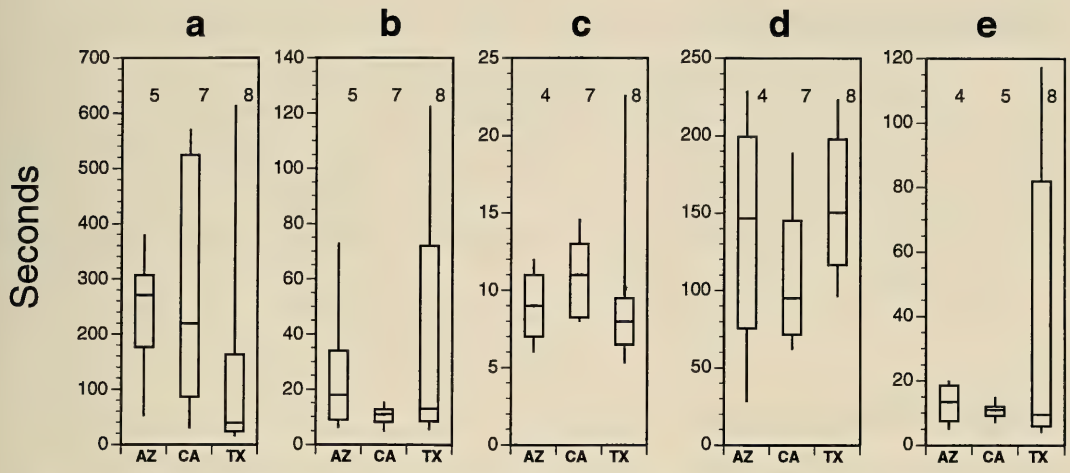

Fig. 1. Duration of courtship events in successful within-population matings. The bar that divides each box is the median value, the top and bottom of the box are the 25% and 75% quartiles and the lines extending beyond the box mark the range of values. The numbers above the boxes are the sample sizes. The figures indicate a, the time until the male mounts the female, b, the duration of precopulatory courtship, c, the duration of copulation, d, the duration of postcopulatory courtship, and e, the time until the female signals receptivity.

allele frequencies of males and females combined.

Results

There was no evidence that courtship behavior differed between the three populations. No unique behaviors were observed in any of the three populations, and there was as much variation found in the sequence and frequency of particular behaviors within populations as between populations. Furthermore, no statistically significant differences between populations were found in the duration of each phase of courtship (Kruskall Wallis test, Fig. 1). For this reason, the description of courtship behavior that follows is based on observations from all three populations.

Courtship behavior.—A male placed in an arena with a female walks rapidly while vigorously antennating the substrate. When a female is encountered the male generally approaches from the side, antennates briefly and attempts to mount. The attempted mounting may be preceded by the male circling the female. Males will attempt to mount walking or motionless females. Females often appear to be arrested upon the approach of a male, with their antennae and abdomen lowered. However, the female may jump away when mounting is attempted; both motionless and moving females have been observed to jump. If the female jumps, the male resumes his search. If the male mounts successfully he stands on the female with his foretarsi on the females head, usually on her eyes, his midtarsi below the wings on the gaster, and his hind tarsi on the female's wings (Figs. 2, 3a).

Precopulatory courtship begins with a relatively long period of antennation and may be accompanied by occasional 'foreleg rubbing,' or one or more wing fans. The end of the precopulatory 'cycle' (sensu van den Assem 1986) is composed of two distinct behaviors: a wing fan and two or three swinging movements of the midlegs. The male then usually backs up to attempt copulation.

Antennation by the male is vigorous, with the mid section of the long, unsegmented male clubs touching the tips of the female antennae (Fig. 3a). Unlike some other chalcidoids, for example the Pteromalinae (van den Assem 1986), female receptivity does not appear to be signaled by

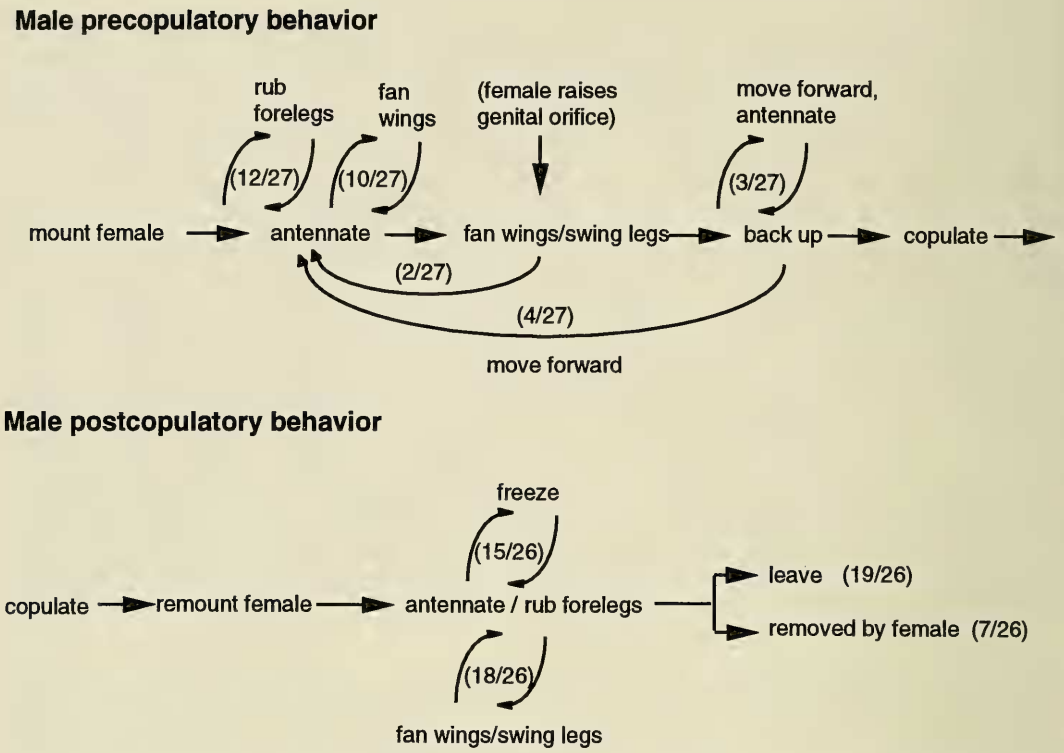

Fig. 2. The sequence of precopulatory and postcopulatory courtship behaviors in successful matings. When a behavior was not universally performed, the fraction of males that performed that behavior is given.

clear changes in antennal position. Female antennal clubs do not usually drop at any point in the cycle. Foreleg rubbing is sometimes a part of precopulatory courtship but is an integral part of postcopulatory courtship (Fig. 2). In this behavior, males lift each of their foretarsi from the females eyes alternately. They may simply retract the leg towards their body and then replace it on the female eye, or perform a more elaborate behavior. In the latter, the tarsus is first crossed under the body until it touches the mesopleuron with the tibia and femur flat against the venter, then the femur-tibia joint is extended downward (Fig. 3b). The femur-tibia joint is then retracted so the leg is again flat across the venter, and the tarsus is drawn back across the body and returned to its original position. This entire action is very rapid, about one second in total duration, and it is difficult to determine the nature of the signal being communicated. This behavior has not been recorded as a part of courtship in parasitic wasps to our knowledge, and we discuss its possible significance below. The end of precopulatory courtship is signaled by a wing fan by the male. This may be a simple flip above the dorsum without extending the wings (Fig. 3c), or the male may also extend the wings outward and down so that they are perpendicular to the long axis of the body and level with the dorsum, and then return them to their resting position. In conjunction with the wing fan or immediately following, the midlegs are swung forward in parallel so that the tarsi pass in front of the females eyes and are then returned (Fig. 3d). The 'leg swing' is usually repeated twice, although some males swing their legs three times and fewer swing their legs once or four times. There is also between-male variation in the nature of the leg swings. Some males swing their legs asynchronously, and

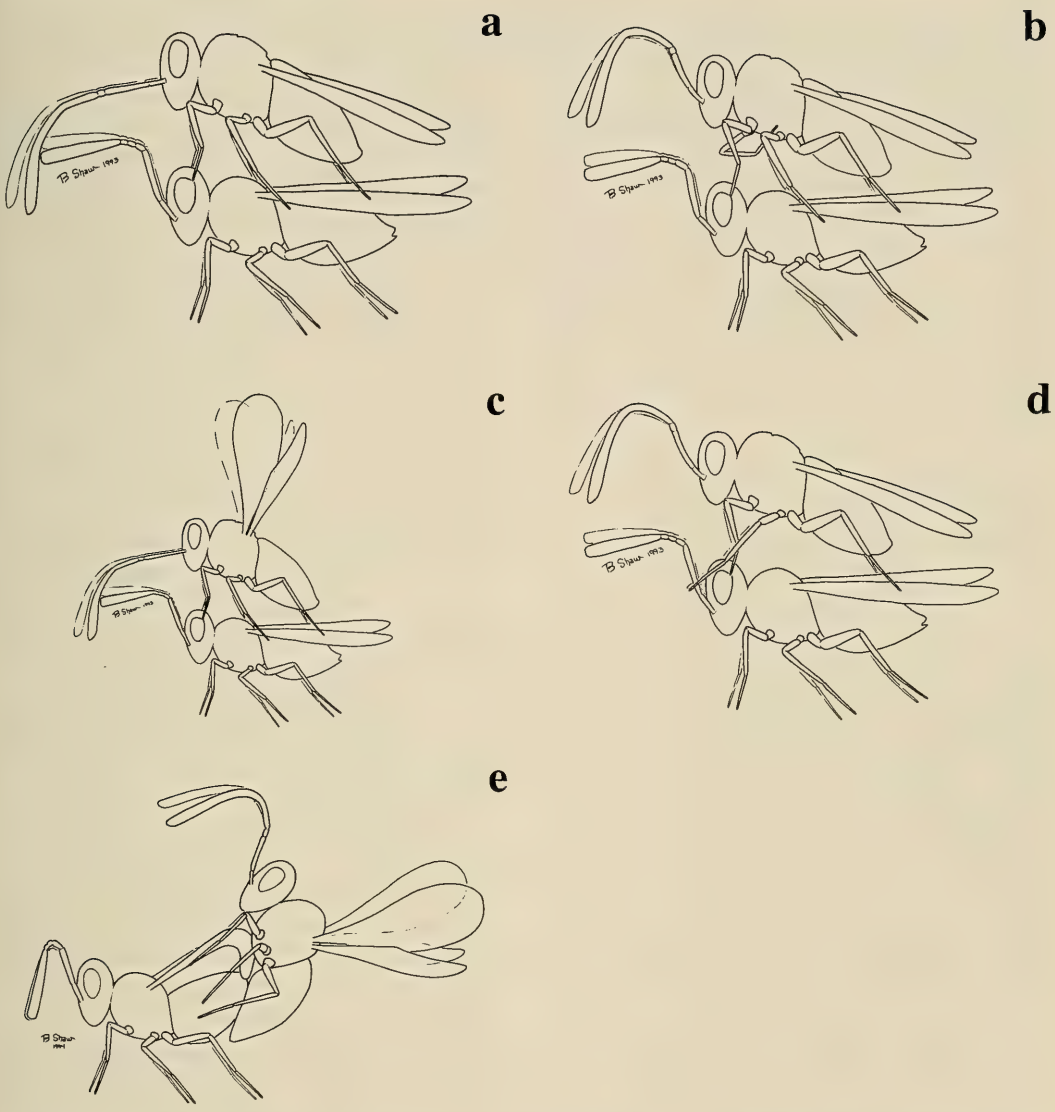

Fig. 3. Courtship behaviors. a, Antennation. b, Foreleg rubbing. c, Wing fanning. d, Midleg swings. e Copulation.

sometimes one of the tarsi hits the female head in the forward swing. Receptive females usually lift the metasoma (exposing the genital orifice) at the time the mid tarsi of the male cross in front of the female eyes for the first or second swing. The mean duration of the precopulatory phase of courtship is 30 seconds ± 9 SE. The leg swings are generally followed by the male backing up to attempt copulation, regardless of whether the female has signaled her receptivity. The exceptions generally occur when the female is actively trying to remove the male; in these instances the male may simply begin the cycle again.

As the male adopts the copulatory position, the female wings are lifted slightly, and flexed downward at the ends by the male. In the male copulatory position the wings serve as a prop against the substrate, the forelegs grip the margins of the wings of the female and the mid and hind legs are

on the female metasoma (Fig. 3e). The duration of copulation is on average 12 seconds ± 1.7 SE, after which the male returns to the mounted position and begins postcopulatory courtship.

Postcopulatory courtship is composed of many of the same behaviors as in the precopulatory cycle, although the frequency and order is different (Fig. 2). Much of the time is spent alternating between antennating and foreleg rubbing or doing both synchronously. Males touch either the middle or distal third of their club to the tip of the females antennae, as in precopulatory courtship; there is no clear pattern in the order or frequency of the two types of antennation. The postcopulatory foreleg rubbing is more pronounced and is performed by all males. Postcopulatory males may 'freeze' and stand motionless on the female. The male may then resume antennation and/or foreleg rubbing, sometimes apparently in response to movements by the female. The wing fan/leg swing behavior is also a frequent component of postcopulatory courtship, but it may be performed at any time and does not appear to signal the end of courtship. Either the male or the female may end postcopulatory courtship; males dismount to the side and walk away at approximately 90° from the female head, while females may dislodge the male after beginning to walk or groom. The duration of postcopulatory courtship (mean 144 seconds ± 11.31 SE) was not significantly different when ended by males compared to females (t = 1.29, df = 24).

The courtship of unreceptive females is characterized by many repetitions of the courtship cycle. While apparently identical to courtship of receptive females initially, the nature of the courtship of unreceptive females is often altered by progressively more strenuous evasive behaviors by the female. Males commonly court far forward of the normal position with their body tilted forward over the female head; reasons for this may include better access to antennae which may be lowered by unreceptive females, and less leverage allowed females that may raise their wings in an apparent attempt to lift the hind legs of the male and tilt him forward and over the female's head. In addition, cycles may be longer as males antennate longer before attempting the wing fan/leg swing behavior. Lastly, the male may not back up after leg swinging if the female is still trying to dislodge him.

Reproductive relationships.—Copulation was never observed between Texas individuals and individuals from either Arizona or California (Table 1). In contrast, there was no significant difference between the frequency of within- and between-population matings in the Arizona and California populations. Interestingly, in reciprocal crosses between Arizona and California, females signaled acceptance more frequently than males succeeded in mating (Table 1). This may have been due to size differences between the two populations (Table 2); in some instances size incompatibility of the male and female appeared to present a physical barrier to mating.

Relatively few of the California and Arizona females reproduced, and those that did had a lower fecundity than did the Texas females (mean no. of progeny, California, 10.50 ± 2.19 SE, n = 6 of 10 females; Arizona, 8.5 ± 4.44 SE, n = 4 of 9 females; Texas, 38.86 ± 7.44 SE, n = 7 of 9 females). The poor reproduction of the Arizona and California females may have been due to the choice of the host plant, collards, for rearing of the F1 progeny. While the Texas population was collected from cabbage, *E.* nr. *californicus* is not generally recovered from *Bemisia* on crucifers in California, even when planted adjacent to plants bearing *Eretmocerus* (Roltsch and Pickett 1994, M. Rose, pers. obs.). These observations suggest there may be differences in host plant affinities between the Texas *Eretmocerus* and the other two populations.

The sex ratio (proportion males) of progeny from crosses between the Arizona and California populations (s.r. = 0.42, n = 33),

Table 1. Summary of within- and between-population mating trials of three populations of *Eretmocerus* sp. nr. *californicus*.

	Trials	Male mounted	Female accepted	Copulation occurred
Within populations				
Texas	10	10	9	9
Arizona	6	5	5	5
California	10	8	7	7
Between populations				
Texas–Arizona	16 (10/6)[1]	13 (10/3)	0***	0***
Texas–California	20 (10/10)	13 (7/6)	0***	0***
Arizona–California	16 (6/10)	11 (5/6)	10 (5/5)	7 (4/3)

[1] The frequencies of the two reciprocal crosses. The first number corresponds to the trial in which females from the population listed first were mated to males of the population listed second.

*** Frequencies in these cells were significantly different (χ^2, 1 df, $P < 0.001$) from the summed frequencies of the two relevant within-population trials.

were no more male-biased than the sex ratios of either the within-population Arizona (s.r. = 0.48, n = 21) or California (s.r. = 0.42, n = 45) crosses. These data suggest there are no postcopulatory reproductive barriers to gene flow between these two populations.

Allozyme analysis.—For two of the five allozymes, there was considerable divergence in allele frequencies between the Texas population and the populations from Arizona and California (Tables 3, 4). The IDH-c allele is more common in the California and Arizona populations while the alternative alleles are more common in the Texas population (Tables 3, 4). Similarly the ME-a allele is more common in the Arizona population, while the ME-b allele is more common in the Texas population (Table 4). MDH, PGI, and PGM showed similar allele frequencies in all populations, although two MDH alleles (MDH-a and MDH-d) were unique to the Texas population (Tables 3, 4). In the Texas population, allele frequencies of the PGI locus changed from the first to the second collection, with the appearance of the PGI-c allele and the disappearance of the PGI-a allele (Tables 3, 4). It is not clear why this between-year difference occurred in the Texas population, but small differences in the time of year collected, geographic location, and collections from a greater variety of host plants in 1994, including cotton and melon as well as cabbage, may have contributed to the difference in allele frequencies at this locus. In all cases, genotype frequencies conformed with Hardy-Weinberg expectation, and allele frequencies of males and females were similar. Samples of the F1 and F2 progeny when compared to parental allozyme patterns supported a Mendelian inheritance pattern for the three loci tested in the 1993 sample.

Genetic distances (both Nei's D and Hillis' D*) estimated from combined gene frequencies of males and females differed be-

Table 2. Comparisons of the lengths of the hind tibiae, in microns.

Population[1]	n	Females[2]	Males
Arizona	19	196 ± 5 SE	187 ± 5 SE
California	20	212 ± 6 SE	197 ± 5 SE
Texas	20	219 ± 4 SE	220 ± 2 SE

[1] Hind tibia length is significantly different among populations ($F_{2,53} = 35.18$, $P < 0.001$), and between Arizona and the other two populations ($F_{1,55} = 20.79$, $P < 0.001$).

[2] Hind tibia length is significantly different between sexes ($F_{1,53} = 4.09$, $P < 0.05$).

Table 3. Allele frequencies from three loci in three populations of *Eretmocerus* nr. *californicus* sampled in 1993. N refers to the number of individuals sampled for each locus.

	Arizona			California			Texas		
	Males	Females	Combined	Males	Females	Combined	Males	Females	Combined
Alleles									
(N)	(16)	(1)		(24)	(14)		(46)	(20)	
IDH-a	—	—	0.000	—	—	0.000	0.087	0.150	0.116
IDH-b	—	—	0.000	0.083	—	0.038	0.870	0.850	0.860
IDH-c	1.000	1.000	1.000	0.917	1.000	0.962	0.043	—	0.023
(N)	(26)	(1)		(34)	(15)		(32)	(21)	
MDH-a	—	—	0.000	—	—	0.000	0.031	0.048	0.041
MDH-b	1.000	1.000	1.000	0.853	0.933	0.891	0.969	0.881	0.919
MDH-c	—	—	0.000	0.147	0.067	0.109	—	0.048	0.027
MDH-d	—	—	0.000	—	—	0.000	—	0.024	0.014
(N)	(27)	(1)		(34)	(15)		(32)	(21)	
PGI-a	—	—	0.000	0.147	0.200	0.172	0.033	0.119	0.083
PGI-b	1.000	1.000	1.000	0.853	0.800	0.828	0.967	0.881	0.917
PGI-c	—	—	0.000	—	—	0.000	—	—	0.000

tween the two electrophoretic runs. When all three populations were examined for three loci, genetic distance (D) between the Arizona and California was only 0.011 while the distances between these two populations (Arizona and California) and Texas, were 0.377 and 0.394, respectively. In the second run, with a larger sample and a greater number of loci, the distance between the Texas and Arizona populations was 0.701. Hillis' D* (1984), which was designed to be less sensitive to variation in gene substitution rates among loci, gave very similar estimates of genetic distance. For the first run, the D* between Arizona and California was 0.010, while the dis-

Table 4. Allele frequencies from five loci in two populations of *Eretmocerus* nr. *californicus* sampled in 1994. N refers to the number of individuals sampled.

	Arizona			Texas		
	Males	Females	Combined	Males	Females	Combined
Alleles (N)	(32)	(43)		(19)	(50)	
IDH-a	—	—	0.000	0.500	0.540	0.539
IDH-b	—	0.035	0.025	0.444	0.410	0.415
IDH-c	1.000	0.965	0.975	0.056	0.050	0.051
MDH-a	—	—	0.000	—	0.020	0.017
MDH-b	1.000	1.000	1.000	1.000	0.960	0.966
MDH-c	—	—	0.000	—	0.010	0.008
MDH-d	—	—	0.000	—	0.010	0.008
ME-a	1.000	1.000	1.000	0.211	0.060	0.084
ME-b	—	—	0.000	0.789	0.940	0.916
PGI-a	0.031	—	0.008	—	—	0.000
PGI-b	0.969	1.000	0.992	0.474	0.270	0.303
PGI-c	—	—	0.000	0.526	0.730	0.697
PGM-a	0.031	—	0.008	—	0.030	0.025
PGM-b	0.594	0.430	0.475	0.474	0.520	0.513
PGM-c	0.375	0.570	0.517	0.526	0.450	0.462

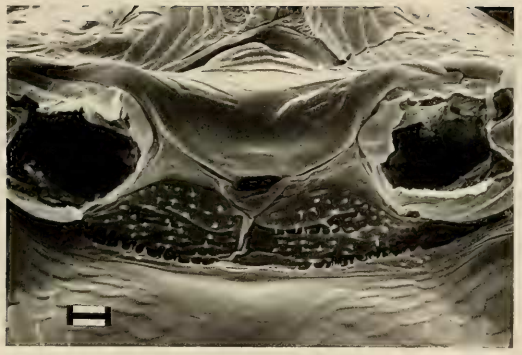

Fig. 4. Scanning electron micrograph (1000×) of epicoxal pads under the forelegs of a Texas *E.* nr. *californicus* male. Scale bar = 10 μm.

tances between Arizona and Texas, and California and Texas were 0.395 and 0.378, respectively. In the second run, the difference between Texas and Arizona was 0.663.

Discussion

Courtship behavior.—Courtship in the three populations of *Eretmocerus* nr. *californicus* shared some attributes with other genera in the Aphelinidae. The courtship behavior of several species of *Aphytis,* a large genus of primary parasitoids of armored scale insects which are also in the Aphelininae (Rosen and DeBach 1979), was thoroughly explored by Gordh and Debach (1978). The precopulatory sequence is abbreviated (3.3 secs on average) and appears to consist only of antennation. The importance of functional antennae in consummating courtship in *Aphytis* was demonstrated by removing the club segments of both males and females; interference with antennal function of either sex prevented copulation (Gordh and DeBach 1978). The courtship stance of male *Aphytis* is similar to *Eretmocerus,* but the midlegs hang free and do not touch the body of the female. Like *Eretmocerus,* females do not appear to signal their acceptance by a change of antennal position, but simply by a change of the metasomal position. Unlike *Eretmocerus,* male *Aphytis* copulate with their hind legs on the substrate. Postcopulatory courtship of *Aphytis* involves antennation, frequent wing fanning and swinging of the mid legs ("semaphoral" movement) in a manner that resembles *Eretmocerus.* The tarsi of *Aphytis* males do not appear to pass very close to the female's eyes, however. While the timing of female acceptance in *Eretmocerus* suggest that females respond to the sight of the tarsi crossing before their eyes, Gordh and Debach (1978) suggest that this movement in *Aphytis* might move pheromones produced by the male thorax. Wing fanning in *Aphytis* is more frequent than in *Eretmocerus* and is performed by males on approach to females as well as during courtship. It is not used in combination with leg swings as it is in *Eretmocerus.* Finally, the foreleg movement observed in *Eretmocerus* is not seen in *Aphytis.* While Gordh and DeBach (1978) might conceivably have missed this very rapid movement without the video recording technology that allows frame by frame observation, we believe the behavior is more likely absent in *Aphytis* because elaborate foreleg movement would seem potentially unstable when the midlegs are not anchored on the female.

The nature of the cues being communicated by foreleg rubbing are unknown but one possibility is that leg movement causes vibrations of some enigmatic structures called the epicoxal pads (Fig. 4). Epicoxal pads appear throughout the subfamily Aphelininae (J. Woolley and M. Hayat pers.

comm.). They were first observed in *Aphytis* by Rosen and DeBach (1979) who suggested that they might have an acoustic function, particularly in courtship. Although the foreleg rubbing of *Eretmocerus* males in courtship would appear to support this idea, there are some problems with this explanation. Females as well as males have epicoxal pads. Furthermore, epicoxal pads are present in taxa such as *Aphytis* where they do not appear to be used in courtship. Performing auditory recordings of *Eretmocerus* courtship would be a first step toward resolving the function of these structures.

Courtship in the more distantly related aphelinid genus *Encarsia* is highly variable; postcopulatory courtship may be absent or persist for up to 26 minutes, and the duration of copulation also varies (Viggiani and Battaglia 1983, Kajita 1989). Leg movements appear to be absent in the species studied, and wing fanning is infrequent; instead antennation appears to replace other behaviors and may involve a number of antennal movements and positions (Viggiani and Battaglia 1983). Like *Aphytis* males, *Encarsia* males support themselves with their hind legs during copulation (Viggiani and Battaglia 1983, Kajita 1989).

Reproductive relationships.—Reproductive incompatibility between populations is generally a result of genetic differentiation of one or more of a suite of behavioral and physiological characters, and is commonly recognized a good indicator of species limits. However, misinterpretation is possible, especially if there are environmental influences on reproductive relationships, or if the presence of continuous variation among several populations is interpreted as discontinuous because only populations at the extremes are tested (Pinto et al. 1991). For this reason, corroboration of reproductive relationships with character systems such as morphology or enzyme patterns is desirable.

In this study the mating trials were clearly supported by the electrophoretic results. The Texas population is distinguished from the other two by two unique MDH alleles, and both IDH and ME show consistent allelic differences between Texas and the others. The genetic distances between the Texas population and populations from Arizona and California are consistent with values commonly found between species of other parasitoid wasps (Kawooya 1983, Unruh et al. 1989, Pinto et al. 1992, Pinto et al. 1993) and among *Drosophila* species (Coyne and Orr 1989). The genetic distances between the Arizona and California populations are more like those found between populations within a species. In concert, these separate lines of evidence support our contention that the Texas *Eretmocerus* is a distinct species from the *Eretmocerus* collected in Arizona and California. These will be described as new species (M. Rose and G. Zolnerowich, in litt.).

Acknowledgments

All determinations were made by Mike Rose. A sample of the individual wasps used in the mating trials were mounted as voucher specimens and deposited in the private collection of Mike Rose. The authors are grateful to Holly Glenn, Kim Hoelmer, Oscar Minkenberg, and Charlie Moomaw for sending field collected material in a very timely manner. Greg Zolnerowich took the SEM micrographs of the epicoxal pads on the Texas *Eretmocerus*. Ty Vaughn assisted with the electrophoretic studies. Ben Shaw illustrated the mating behavior from video stills. We also express our gratitude to Ray Carruthers for his encouragement and support on this project, and to Jim Woolley for his thoughtful comments on the manuscript.

Literature Cited

Bartlett, A. C. and N. J. Gawell. 1993. Determining whitefly species. Science 261: 1333–1334.

Bellows, T. S. J., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Annals of the Entomological Society of America 87: 195–206.

Brown, J. K., D. R. Frohlich, and R. C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: Biotypes

of *Bemisia tabaci* or a species complex? Annual Review of Entomology 40: 511–534.

Campbell, B. C., J. E. Duffus, and P. Baumann. 1993. Determining whitefly species. Science 261: 1333–1334.

Coudriet, D. L., D. E. Meyerdirk, N. Prabhaker, and A. N. Kishaba. 1986. Bionomics of sweetpotato whitefly (Homoptera: Aleyrodidae) on weed hosts in the Imperial Valley, California. Environmental Entomology 15: 1179–1183.

Coyne, J. A. and H. A. Orr. 1989. Patterns of speciation in *Drosophila.* Evolution 43: 362–381.

Gerling, D. 1966. Studies with whitefly parasites of Southern California. I. *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae). Canadian Entomologist 98: 707–724.

Gordh, G. and P. DeBach. 1978. Courtship behavior in the *Aphytis lingnanensis* group, its potential usefulness in taxonomy, and a review of sexual behavior in the parasitic Hymenoptera (Chalcidoidea: Aphelinidae). Hilgardia 46: 37–75.

Hillis, D. M. 1984. Misuse and modification of Nei's genetic distance. Systematic Zoology 33: 238–240.

Howard, L. O. 1895. Aphelinidae of North America. United States Department of Agriculture Technical Series 1. 44 pp.

Kajita, H. 1989. Mating and oviposition of three *Encarsia* species (Hymenoptera: Aphelinidae) on the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). Applied Entomology and Zoology 24: 11–19.

Kawooya, J. K. 1983. Electrophoretic discrimination of species of the *Muscidifurax* complex. Ph.D. Thesis, University of Illinois, Urbana, IL. 114 pp.

Kazmer, D. J. 1991. Isoelectric focusing procedures for the analysis of allozymic variation in minute arthropods. Annals of the Entomological Society of America 84: 332–339.

McAuslane, H. J., F. A. Johnson, and D. A. Knauft. 1994. Population levels and parasitism of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on peanut cultivars. Environmental Entomology 23: 1203–1210.

Nei, M. 1972. Genetic distance between populations. The American Naturalist 106: 283–292.

Pinto, J. D., D. J. Kazmer, G. R. Platner, and C. A. Sassaman. 1992. Taxonomy of the *Trichogramma minutum* complex (Hymenoptera: Trichogrammatidae): Allozymic variation and its relationship to reproductive and geographic data. Annals of the Entomological Society of America 85: 413–422.

Pinto, J. D., G. R. Platner, and C. A. Sassaman. 1993. Electrophoretic study of two closely related species of North American *Trichogramma*: *T. pretiosum* and *T. deion* (Hymenoptera: Trichogrammatidae). Annals of the Entomological Society of America 86: 702–709.

Pinto, J. D., R. Stouthamer, G. R. Platner, and E. R. Oatman. 1991. Variation in reproductive compatibility in *Trichogramma* and its taxonomic significance (Hymenoptera: Trichogrammatidae). Annals of the Entomological Society of America 84: 37–46.

Polaszek, A., G. A. Evans, and F. D. Bennett. 1992. *Encarsia* parasitoids of *Bemisia tabaci* (Hymenoptera: Aphelinidae, Homoptera: Aleyrodidae): A preliminary guide to identification. Bulletin of Entomological Research 82: 375–392.

Roltsch, W. J. and C. H. Pickett. 1994. Silverleaf whitefly natural enemy refuges in Imperial County, p. 146. *In* Henneberry, T. J., N. C. Toscano, R. M. Faust, and J. R. Coppedge, eds., Silverleaf Whitefly. 1994 Supplement to the Five-Year National Research and Action Plan, Orlando, FL. USDA/ARS Publ. 125.

Rosen, D. 1986. The role of taxonomy in effective biological control programs. Agriculture, Ecosystems and Environment 15: 121–129.

Rosen, D. and P. DeBach. 1973. Systematics, morphology and biological control. Entomophaga 18: 215–222.

———. 1979. Species of *Aphytis* of the world (Hymenoptera: Aphelinidae). Israel University Press, Jerusalem, and W. Junk. The Hague. 801 pp.

Swofford, D. L. and R. B. Selander. 1989. BIOSYS-1. A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Release 1.7. Urbana, IL.

Unruh, T. R., W. White, D. Gonzalez, and J. B. Woolley. 1989. Genetic relationships among seventeen *Aphidius* (Hymenoptera: Aphidiidae) populations, including six species. Annals of the Entomological Society of America 82: 754–768.

van den Assem, J. 1986. Mating behaviour in parasitic wasps, pp. 137–167. *In* Waage, J. K. and D. J. Greathead, eds., Insect Parasitoids. Academic Press, London.

Viggiani, G. and D. Battaglia. 1983. Courtship and mating behavior in a few Aphelinidae (Hym. Chalcidoidae). Bollettino del Laboratorio di Entomologia agraria Filippo Silvestri 40: 89–96.

Wharton, R. A., J. B. Woolley, and M. Rose. 1990. Relationship and importance of taxonomy to classical biological control, pp. 11–15. *In* Habeck, D. H., F. D. Bennett, and J. H. Frank, eds., Classical Biological Control in the Southern United States. Bulletin no. 355, Southern Cooperatives Series.